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MECHANISM FOR DISORDERS IN PLASTIC PROCESSES
IN TISSUE IN PROLONGED HYPODYNAMIA

by

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Protein synthesis intensity was studied in the subcellular structures of the myocardium, skeletal muscles, liver and kidneys of adult rats subjected to hypokinesia (in immobilization chambers) for 15, 30 and 45 days. An anabolic agent (retabolil) and vitamin D (a Ca metabolism regulator) were administered to two groups of rats. In the second week of hypokinesia inhibition of synthesis processes was observed. Administration of retabolil increased protein synthesis both in the normal and hypokinesia-subjected rats; however, in the latter group, synthesis did not completely normalize, especially in the myocardium. Administration of vitamin D also stimulated protein synthesis, apparently by normalizing Ca tissue metabolism. The combined action of both preparations was the most effective in normalizing protein synthesis intensity. It was concluded that inhibition of synthesis is related to weakening of hormone synthesis induction and disorder of Ca metabolism.

Prolonged limited motor activity accompanying long space flights as a result of release during weightlessness of the pose (maintaining a pose) musculature, as well as the complicating clinical course of many diseases (myocardial infarct, affections of the nervous system, certain types of trauma, and

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*Numbers in margin indicate pagination in original foreign text.

others), recently has been studied in different aspects. It has been shown that the negative effect of hypodynamia is manifest in pronounced change in the tissue metabolism: disorder of the water-saline metabolism, especially losses of Ca by the organism [3,10], reduction in the hormonal activity [11], considerable drop in the level of oxidizing phosphorylation [3,4] and restructuring of the processes of energy formation [6], decrease in the synthesis of tissue proteins and increase in their breakdown [20]. Weakening of the reparative processes produces functional-structural disorders of the internal organs, decrease in their weight, reduction in the mass of muscle tissue, which sharply limits the performance capacity of the organism [4,18].

The pathogenetic mechanisms for plastic (synthetic) processes in the tissues during hypodynamia are unknown, and to clarify them, in the opinion of V. V. Parin et al. [9] it is theoretically justified to use resources that act on the regulation apparatus of the protein synthesis and cation metabolism. Taking into account the significant role of anabolic hormones and cations in regulating the protein metabolism of tissues [4,5,12] we studied the intensity of protein synthesis in the subcellular structures of the myocardium of skeletal muscles, liver and kidneys of rats during hypodynamia on the background of the effect of an anabolic agent (retabolil) and a preparation that regulates the Ca metabolism in the organism--ergocalciferol (vitamin D). /42

Technique

The experiments were conducted on mature male rats weighing 170-240 g. Hypodynamia was created by maintaining the animals in individual universal immobilization chambers [8] that limit movement in all directions, for 15, 30 and 45 days. The rats of one group on the background of considerable disorder in the

protein synthesis for the last 7 days, daily were administered ergocalciferol intra-abdominally 100 ME each, the other 2 weeks before death twice with a 7-day interval were administered intra-muscularly--anabolic steroid of prolonged action, retabolil in a dose of 1 mg/kg. The intensity of the protein synthesis in the mitochondria and post-mitochondrial supernatant of tissues was judged by the inclusion of glycine-1-C¹⁴ administered intra-abdominally in 25 μ curie/100 g 6 hours before death. The mitochondria were isolated by the method of differential centrifuging [13] in 0.25 M of sucrose solution (pH 7.4) with 0.001 M of EDTA. The protein of the mitochondria and the supernatant fluid was precipitated by a 10% solution of trichloroacetic acid, purified with successive reprecipitation with 5% solution, washing with alcohol and ether, and drying at 60° for 12 hours. The radioactivity of the preparations was measured on the end counter BFL-T-25 with conversion unit PP-16. The specific activity was expressed in impulses per minute per 10 mg of protein.

Results and Discussion

The findings are presented in the table.

Prolonged hypodynamia resulted in considerable suppression of the inclusion of glycine-1-C¹⁴ in the proteins of the mitochondria and the supernatant fluid of the myocardial tissues (respectively by 47.8 and 46.4%) and the skeletal muscles (by 46.4 and 47%). Suppression of the synthetic processes in the tissues was manifest already from the second week of the experiment. The early emerging suppression of protein synthesis in the mitochondria, probably, is one of the reasons, as Ye. A. Kovalenko et al. established [3,4], for the disturbance in the processes of oxidation and phosphorylation in the tissues at the later stages of hypodynamia even on the background of intensified oxygen consumption by the homogenates of certain

tissues, i.e., deficit in synthesis of macroergs, which can result in the future in energy limitation of the actual synthetic processes. Apparently, one should consider the phase intensification in the power of glycolysis in maintaining the energy potential of the tissues that we found [6] to be compensatory in the dynamics of hypodynamia.

Reduction during hypodynamia in the rate of protein synthesis of muscle tissues corresponds to the modern ideas on the dependence of protein synthesis on the level of the physiological function of the cell [7]. However, the reduction observed here in the incorporation of marked amino acid into the proteins of the organelles, especially supernatant fluid of the liver and kidneys (see table) indicates the serious disorders in the apparatus for controlling protein synthesis. Here in certain tissues an increase was found in the ratio of histon/DNA[15], reduction in the RNA content [1]. Apparently, the prolonged immobilization as a consequence of a deficit in proprioceptive afferentation and attenuation in hormonal induction of the protein-synthesizing processes changes all the levels of integration of the organism, in particular the cellular and molecular, and inhibition of transcription can be the primary mechanism for depression of the protein synthesis during hypodynamia.

For the purposes of verifying the given hypothesis we used retabolil whose molecular mechanism of anabolic action, as other hormones [17], consists of its penetration into the cellular nucleus and interaction with the histons, which results in the increase in the matrix activity of chromatin and RNA-polymerase, activation of transcription and initiation of protein synthesis in the polysomes. Under the influence of retabolil the rate of glycine- ^{14}C inclusion in the proteins of the mitochondria and supernatant fluid of the studied tissues considerably rose in the

intact animals. A more pronounced intensification of synthetic processes here was observed in the cytostructures of the myocardium and kidneys--tissues with pronounced dominance of the oxidizing nature of metabolism. In the rats with hypodynamia /44 retabolil significantly stimulated the protein synthesis in the subcellular structures of the myocardium and almost normalized this process in the other tissues. It was shown [10] that the anabolic hormones produce during hypodynamia also an inhibition of the fluid and Ca salts in the organism, i.e., have a correcting effect in the water-saline metabolism, one of the most critical links in the hypodynamic disorders.

The absence of complete normalization of the synthetic processes, in particular in the myocardium, in the experimental animals during the effect of retabolil indicates the change here in other regulating (probably, electrolyte) factors of the nuclear-cytoplasmic interrelationships in the process of the life activity of the cell. In particular, the high Ca^{++} gradient on the mitochondrial membrane is necessary for ATP synthesis, and possibly plays a regulatory role in the processes of energy utilization of the respiratory chain of mitochondria [21]. Since during hypodynamia Ca utilization is reduced and its removal from the organism is intensified [3,10] in the next series of experiments we studied the effect on the protein synthesis processes in the tissues of ergocalciferol (vitamin D), which, like the products of its auto-oxidation, not only block the Ca loss by the organism, but also increase its assimilation by the tissues [2,16] by means of active transport through the cellular membranes [22].

In the intact animals with undisturbed Ca metabolism the ergocalciferol significantly did not alter the rate of inclusion of marked amino acid into the subcellular fractions of the muscles and kidneys, only in the supernatant fluid of the kidney tissue was a trend towards its increase observed. However, in

INTENSITY OF INCLUSION OF GLYCINE-1-C¹⁴ (in imp/min/10 mg protein) IN PROTEINS OF CYTOSTRUCTURES OF RAT TISSUES DURING HYPODYNAMIA ON THE BACKGROUND OF THE EFFECT OF RETABOLIL AND ERGOCALCIFEROL

Content of experiment	Myocardium				Skeletal muscle				Liver				Kidneys			
	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant	mito-chondria	super-natant
Control—Intact rats (6)	360±21,6	306±25,8	140±13,1	68±3,9	700±35,5	727±43,5	531±29,3	502±34,7								
Hypodynamia 15 days (5)	272±9,3 <0,01	280±17,8 <0,001	100±6,3 <0,05	55±3,3 <0,001	674±47,6 40±2,5	795±54,0 737±49,5	546±39,3 <0,05	438±41,1 <0,05								
Retabolil 1 (5)	230±12,1 <0,001	216±16,8 <0,05	91±5,8 <0,01	40±2,5 <0,001	737±49,5 36±2,8	610±19,5 <0,01	500±49,1 482±37,2	385±16,2 <0,05								
Hypodynamia 30 days (6)	D	188±16,4 <0,001	161±11,6 <0,05	75±4,2 <0,01	725±47,1 92±4,1	482±37,2 948±45,3	423±26,2 690±40,7	370±22,5 722±17,6								
Hypodynamia 45 days (6)	D	494±32,5 <0,01	462±27,8 <0,01	167±8,1 <0,01	755±46,4 92±4,1	755±46,4 <0,01	948±45,3 815±34,8	690±40,7 540±23,2								
Retabolil 1 (5)	D	367±25,3 <0,01	318±31,4 247±15,8	144±13,9 115±6,7	75±8,7 60±5,0	728±56,9 794±59,2	815±34,8 675±36,1	562±27,3 548±34,9								
Ergocalciferol (6)	D	265,4±16,6 <0,01	217±12,6 <0,01	106±6,4 <0,01	106±6,4 <0,01	724±36,1 51±3,0	590±21,3 512±35,5	455±21,1 <0,05								
Hypodynamia 45 days + ergocalciferol (8)	D ₁	234±16,4 <0,001	242±12,6 <0,05	106±6,4 <0,05	106±6,4 <0,01	724±36,1 787±51,1	590±21,3 708±49,1	455±21,1 <0,05								
Hypodynamia 45 days + betabolil and ergocalciferol (10)	D ₁	321±26,9 0,01	270±21,6 <0,001	120±12,0 <0,01	62±7,8 <0,01	787±51,1 708±49,1	550±43,1 550±43,1	498±44,1 <0,01								

Note: D—reliable differences with control, D₁—with indices in 45-day hypodynamia.
In parentheses—number of animals.

rats with hypodynamia under the influence of ergocalciferol the rate of protein synthesis rose in the cytostructures of both the myocardium and the skeletal muscles, as well as the liver and kidneys, apparently, due to the correction of Ca metabolism in the tissues [16] and subsequent normal functioning of the enzyme systems closely linked to the membranes of the cellular organelles [14]. Apparently, the optimal Ca concentration in the tissues is a necessary factor in the process of protein synthesis. The effect of ergocalciferol (vitamin D) on Ca metabolism is governed by induction of DNA-dependent RNA synthesis and subsequent formation of a specific protein component in the system of Ca transport, since the use of $1,2-\text{H}^3$ -vitamin D₃ reveals its cumulation in the nuclear fraction, while actinomycin D suppresses the stimulating effect of vitamin D on RNA synthesis [19]. Probably the basis for the disorders in calcium metabolism during hypodynamia is the blockade of protein synthesis by the Ca transport system through the biological membranes, which in turn intensifies the depression of synthetic processes.

The relative normalization of intensity in protein synthesis in the organelles of different tissues during hypodynamia was observed with the simultaneous effect of the protein synthesis inducers and regulators of calcium metabolism (see table).

Thus, during hypodynamia the combined, mutually-governed disruption in calcium and plastic metabolism is observed which opens up the outlook for directed preventive influence on the organism during forced hypodynamia.

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